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ACCESSION NUMBER: 1996:412038 BIOSIS DOCUMENT NUMBER: PREV199699134394

TITLE: Conditions favoring RNA polymerase I transcription in

permeabilized cells.

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SOURCE: Experimental Cell Research, (1996) Vol. 226, No. 1, pp.

114-125.

ISSN: 0014-4827.

DOCUMENT TYPE: Article
LANGUAGE: English

RNA synthesis can be detected in nuclei using modified RNA precursors (Br-UTP) introduced in permeabilized cells. Surprisingly, RNA pol I transcripts are detected only after inhibition of RNA pol II or salt enhancement of RNA pol I activity. By modifying a previously reported protocol, we found that RNA pol I transcripts can be detected selectively or simultaneously with RNA pol II transcripts without any drug treatment. Removing glycerol from the permeabilization and transcription buffers and improving the permeabilization using Triton X-100 revealed RNA pol I transcription in two cell lines (mammalian and Xenopus) and in isolated mouse oocytes. The transcripts were most probably rRNA because they were detected in the nucleoli, digested by RNase, sensitive to actinomycin D, and resistant to a-amanitin. We found by microinjection of the Br-UTP precursors in living cells that low ionic strength allows the detection of RNA pol I transcription. Electron microscopy of mouse oocytes showed that the "looseness" of the nucleolar organization is associated with the detection of the RNA pol I transcription; this detection does not necessarily need nucleolar disorganization. The data obtained with both permeabilized cells and microinjections of RNA precursors in the absence of glycerol support the hypothesis that the degree of hydration of the cell plays a role in RNA pol I transcription.

QH 583.69